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CHARACTERISTICS OF *Valdensia heterodoxa* Peyr. AS AN *Ericaceae* PATHOGEN IN POLAND

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ABSTRACT

Valdensia heterodoxa as a parasitic fungus was observed on *Ericaceae* family plants i.e. blueberry (*Vaccinium corymbosum* L.), bilberry (*V. myrtillus* L.) and lingonberry (*V. vitis-idaea* L.). Isolates of this polyphagous were obtained from the infected leaves of different cultivars of highbush blueberry collected from commercial plantation in Mazovia province and from bilberry collected from forests in Pomerania province. PCR amplification of selected rDNA fragments (ITS1, 5.8S, ITS2) was done with ITS1F and ITS4A primers. Bioinformatic analysis revealed similarity 99–100% between selected nucleotide sequences of *V. heterodoxa* isolates from bilberry and highbush blueberry. The sequences of bilberry isolates were obtained and described for the first time in Poland. Their reference sequence was deposited in GenBank (KT121733). In laboratory experiments conidia of selected bilberry isolates on OA medium were 278 ±6 × 140 ±4 µm. Conidia from highbush blueberry, bilberry, and lingonberry were measured. Depending on the host plant conidia were different in the length of the arms and width of the head. The growth of the fungal isolates on PDA (potato dextrose agar), OA (oatmeal agar), WOA (weak oatmeal agar), SNA (salt nutrient agar) media was examined. The cultures were divided into two groups based on their morphology on PDA medium.

Key words: *Vaccinium corymbosum*, valdensia leaf blight, bilberry, PCR identification

INTRODUCTION

Valdensia heterodoxa Peyr. is a parasitic fungus that causes valdensia leaf blight. It is classified in the phylum *Ascomycota*, class *Leotiomycetes*, subclass *Leotiomycetidae*, order *Helotiales*, and family *Sclerotiniaceae* [Kirk et al. 2008]. In 1953 the fungus was observed and described for the first time by Peyronel [1923] in Italy. In the sexual stage (teleomorph) it develops apothecia in which ascospores are produced in asci. Apothecia arise from sclerotia on infected veins of overwintered leaves [Redhead 1974]. The asexual stage (anamorph) of the pathogen was observed 30 years before the teleomorph was found, i.e. in 1923. The conidia of this fungus are large, stellate, multicellular propagules that can discharge from leaves to a height of ~20 cm [Redhead and Perrin 1972a, b, Norvell and Redhead 1994, Mułenko and Woodward 1996, Zhao and Shamoun 2010].

The ability of the fungus to infect plants largely depends on environmental conditions: high humidity and temperature between 15 and 20°C [Hildebrand and Renderos 2007, Annis and Yarborough 2009]. Disease symptoms of *V. heterodoxa* include round or oval brown or almost black circular zonated necrosis

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(diameter up to 1 cm) surrounded by a dark-brown border. It is formed due to local production of anthocyanin pigment which limits the development of the fungus *V. heterodoxa* [Peyronel 1923, Redhead and Perrin 1972a, b].

The range of fungus host plants is very wide and includes many various plant species inhabiting forests and other plant communities from many geographic zones. Previously, the pathogen was identified in Europe, North America, and Asia [Redhead 1974, Mułenko and Woodward 1996, Mułenko et al. 2008]. The most commonly infected species include plants from the family *Ericaceae*, e.g. the bilberry (*V. myrtillus* L.), salal (*Gaultheria shallon* Pursh.) and other plants of the genus *Vaccinium* [Aamlid 2000]. The oldest data about the distribution of the *Valdensia heterodoxa* asexual stage in Poland originate from the beginning of the 20th century. This fungus was found on leaves of shrubs, trees and herbaceous plants growing in the Białowieża Primeval Forest [Siemaszko 1929, 1933, 1934]. In Białowieża National Park fungus *V. heterodoxa* was present on: lily of the valley (*Convallaria majalis* L.), wood sorrel (*Oxalis acetosella* L.), Solomon's seal (*Polygonatum multiflorum* (L.) All.), viper's grass (*Scorzonera humilis* L.), mountain ash (*Sorbus aucuparia* L.), chickweed wintergreen (*Trientalis europaea* L.), bilberry (*Vaccinium myrtillus* L.) and lingonberry (*V. vitis-idea* L.) [Faliński and Mułenko 1992 according to Mułenko and Woodward 1996]. In 2011 the pathogen was found on highbush blueberry plants cv. Bluecrop in one of nursery pots in Mazovia province as well [Dzięcioł et al. 2014].

The aim of the study was to confirm the identity of the fungus causing leaf blight of a few cultivars of highbush blueberry (grown in commercial plantation) and bilberry (grown in forests) and to provide and compare morphological and genetic characteristics of its isolates to reference isolates.

MATERIALS AND METHODS

The study was carried out from 2012 to 2015. The observations were conducted on a few commercial plantations of highbush blueberry located in selected regions of Poland: Mazovia (8 plantations), Łódź (5 plantations), Lublin (2 plantations), and Podlasie (3 plantations). Bilberry and other common plants like: lingonberry, lily of the valley and mountain ash growing around commercial plantations in the aforementioned locations and in parks or forests in Pomerania (3 locations) and Lublin (4 locations) provinces were examined as well.

Plant leaves with visible symptoms of valdensia leaf blight were collected during lustration. To obtain isolates of *V. heterodoxa*, sections (2–3 mm) from the border of healthy and diseased tissue from the leaf spot were cut out and disinfected with 1% sodium hypochlorite. Next, they were transferred into 10 cm Petri dishes with PDA. The plates were kept a few day at 19°C in daylight. Hyphae fragments were transferred into new dishes with PDA. The obtained isolates were used to identify the pathogen and create a collection on PDA slants, which were stored at 4°C.

Forty isolates of *Valdensia heterodoxa* were examined. As reference isolates Val 1, 2, 3A, 3B, 3C, 3D, 4 and 5 were used. They originated from a collection of the Department of Plant Pathology, Warsaw University of Life Sciences – SGGW in Warsaw and were obtained in 2011 from highbush blueberry cv. Bluecrop and described by Dzięcioł et al. [2014].

All isolates were tested for pathogenicity. To fulfill Koch's postulates, 4 leafy shoots that had been cut from healthy plants both of highbush blueberry cv. Bluecrop or bilberry were used. They were placed in glass Erlenmeyer flasks filled with water. Prior to inoculation, the leaves were disinfected with a 1% sodium hypochlorite. Inoculation was conducted using 5-mm-in-diameter media discs overgrown with mycelium. They were cut out with a sterile cork borer from 20-day-old cultures of fungal isolates grown on PDA medium in Petri dishes. After inoculation the leaves were sprayed with sterile distilled water using an atomizer, placed in plastic bags and sealed. Incubation was carried out at 19°C. The leaves were examined 5 days after the inoculation. In the next step,

re-isolates were obtained and compared to isolates used for inoculation.

For molecular identification of *V. heterodoxa* the same isolates as for the pathogenicity test were used. The cultures grown on PDA in Petri dishes were incubated for 10 days at 19°C in daylight. For extraction of genomic DNA the mycelium from the medium surface was scraped with a sterile scalpel and triturated in a mortar in the presence of liquid nitrogen. Isolation of DNA was carried out using the Wizard Genomic DNA Purification Kit (Promega Corporation), according to the manufacturer's protocol. Amplification of rDNA fragments (ITS1, 5.8S, ITS2) was done with primer pair ITS1F and ITS4A [Larena et al. 1999]. PCR amplification was performed according to Nekoduka et al. [2012] with an annealing temperature of 57°C (instead of 50°C) and a modified number of cycles (28 cycles instead of 30) (Applied Biosystems Veriti 96 Well Thermal Cycler). Amplified fragments were separated electrophoretically in 1.2% agarose / TBE gels in the presence of ethidium bromide. The amplified fungal DNA fragments were sequenced in the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. The sequences were analyzed and compared with each other using ClustalW2 [http://www.ebi.ac.uk/Tools/msa/clustalw2/]. They were also aligned to the existing sequence of this fungus in GenBank using the BLAST program [http://www.ncbi.nlm.nih.gov/BLAST].

Identification of the fungus using traditional methods was performed by means of the available mycological keys [eg. Kochman and Majewski 1970, Arx 1974, Kochman 1986, Marcinkowska 2012] and original publications [Zhao and Shamoun 2010, Nekoduka et al. 2012]. Leaves collected from plants of highbush blueberry and bilberry infected by *V. heterodoxa* were examined under an stereoscopic microscope (Olympus SZ11). The conidia and hyphae of the fungus were observed under a light microscope (Olympus BX50) equipped with a DP71 camera and the Cell F program (Olympus). Fifty conidia isolated from each of the plants were measured (width of the head and length of the arms). Photographic documentation was performed using a digital camera Canon PowerShot G3 and Olympus E410.

The obtained and reference isolates of *V. heterodoxa* fungus were divided into groups according to their morphological features (colour and texture of mycelium) on PDA medium (tab. 1). The growth of selected isolates of the fungus representative for each group (group I: Val. 3A,Vh/Bg/4, Vh/Bg/5, Vh/Un/4, Vh/Un/6 from highbush blueberry, VH/J/1, VH/J/11 from bilberry and group II: Val. 4, Vh/Bg/3, Vh/Bg/6, Vh/Un/1 from highbush blueberry, VH/J/2 and VH/J/14 from bilberry) was examined on the following media: PDA, OA, WOA and SNA. Discs (diameter 5 mm) were cut out with a sterile cork borer from 20-day-old cultures of thirteen isolates grown on PDA medium. Next, they were transferred to the central part of each medium in Petri dishes (diameter 10 cm, 10 dishes per isolate). The cultures were incubated in the laboratory in daylight at 19–20°C. The observations were conducted for 45 days. Then colony diameters were measured and sporulation of the fungus was observed. The results of the measurement of the mycelium diameter were used for calculation of linear growth of fungal colonies.

Furthermore, the range of host plants of the fungus was examined. Observations were conducted in parks and forests on bilberry, lingonberry, solomon's seal, lily of the valley, mountain ash, cornel-tree (*Cornus* L.) and tree peony (*Paeonia suffruticosa* Andrews). Leaves with symptoms of the disease were collected. Then observations of the pathogen signs on the spots were carried out under a light microscope. From each plant species infected by *V. heterodoxa* 50 conidia were measured and compared with the size of conidia developed on highbush blueberry. The results were statistically analysed (Statgraphics Plus 4.1). Observations and measurement of conidia, photographic documentation were performed using the same equipment as mentioned above.

Table 1. The growth of selected isolates of the fungus representative for each group

No.	Name of isolat	Origin			Group
		Plant species	Region/ kind of plantations or forest	Geographical coordinates	
1	2	3	4	5	6
1	Vh/Bg/1	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
2	Vh/Bg/2	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
3	Vh/Bg/3	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	Second group (II)
4	Vh/Bg/4	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
5	Vh/Bg/5	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
6	Vh/Bg/6	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	Second group (II)
7	Vh/Bg/7	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
8	Vh/Bg/8	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
9	Vh/Bg/9	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
10	Vh/Bg/10	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
11	Vh/Bg/11	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
12	Vh/Bg/12	Highbush blueberry cv. Bluegold	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	Second group (II)
13	Vh/Un/1	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	Second group (II)
14	Vh/Un/2	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
15	Vh/Un/3	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
16	Vh/Un/4	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
17	Vh/Un/5	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
18	Vh/Un/6	Highbush blueberry cv. unknown	Mazovia provinces / crop plantations	51°55'43"N 20°59'39"E	First group (I)
19	Vh/J/1	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	First group (I)
20	Vh/J/2	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	Second group (II)

1	2	3	4	5	6
21	Vh/J/3	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	Second group (II)
22	Vh/J/4	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	First group (I)
23	Vh/J/5	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	First group (I)
24	Vh/J/6	Bilberry	Lublin provinces / forest	51°30'25"N 23°31'17"E	First group (I)
25	Vh/J/7	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
26	Vh/J/8	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	Second group (II)
27	Vh/J/9	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
28	Vh/J/10	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
29	Vh/J/11	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
30	Vh/J/12	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
31	Vh/J/13	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
32	Vh/J/14	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	Second group (II)
33	Vh/J/15	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
34	Vh/J/16	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
35	Vh/J/17	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
36	Vh/J/18	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
37	Vh/J/19	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
38	Vh/J/20	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
39	Vh/J/21	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	First group (I)
40	Vh/J/22	Bilberry	Pomerania provinces / forest	54°49'54"N 18°12'35"E	Second group (II)
41	*Val. 1	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)
42	*Val. 2	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	Second group (II)
43	*Val. 3A	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)

1	2	3	4	5	6
44	*Val. 3B	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)
45	*Val. 3C	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)
46	*Val. 3D	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)
47	*Val.4	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	Second group (II)
48	*Val.5	Highbush blueberry cv. Bluecrop	Mazovia provinces / Nursery plantations	51°56'24"N 20°57'16"E	First group (I)

* reference isolates

Fig. 1. Brown, necrotic, zonated spots on leaves of highbush blueberry cv. Bluegold

Fig. 2. Symptoms of *V. heterodoxa* on bilberrys' leaves

RESULTS

During the four years of our studies of eighteen plantations of highbush blueberry and seven, natural stands the symptoms of valdensia leaf blight were observed a few times. In Mazovia province the pathogen was noted on highbush blueberry cv. Bluegold and two unknown cultivars in 2013 and 2014 (fig. 1). In the natural stands the pathogen was observed commonly each year on wild-growing bilberry in forests in: Woroblin and Okuninka (Lublin province),

Fig. 3. Symptoms of *V. heterodoxa* on lingonberrys' leaves

in Karwia (Pomerania province), and Hajnówka and Bialowieża (Podlasie province). The disease symptoms were round or oval, brown or almost black circular zonated necroses surrounded by dark-brown borders (fig. 2). The same type of symptoms were observed on lingonberry too (fig. 3). On the lower side of the leaves, in the central part of each spot, large, star-shaped conidia were observed.

During our research forty isolates of *Valdensia heterodoxa* were obtained. Eighteen isolates originating from leaves of highbush blueberry cv. Bluegold (Vh/Bg/1-12) and two unknown cultivars (Vh/Un/1-6) and twenty two isolates from bilberry shrubs (Vh/J/1-22). Unfortunately, no isolate was obtained from lingonberry plants but symptoms of the disease with typical fungus conidia of this species were observed. Koch's postulates for all isolates were positive. Five days after the inoculation of highbush blueberry and bilberry the first lesions were observed. Leaves from highbush blueberry were infected with highbush blueberry isolates as well as with bilberry isolates. These symptoms were similar to those on infected plants growing on the plantation. Re-isolates of the fungus were the same as those used for inoculation.

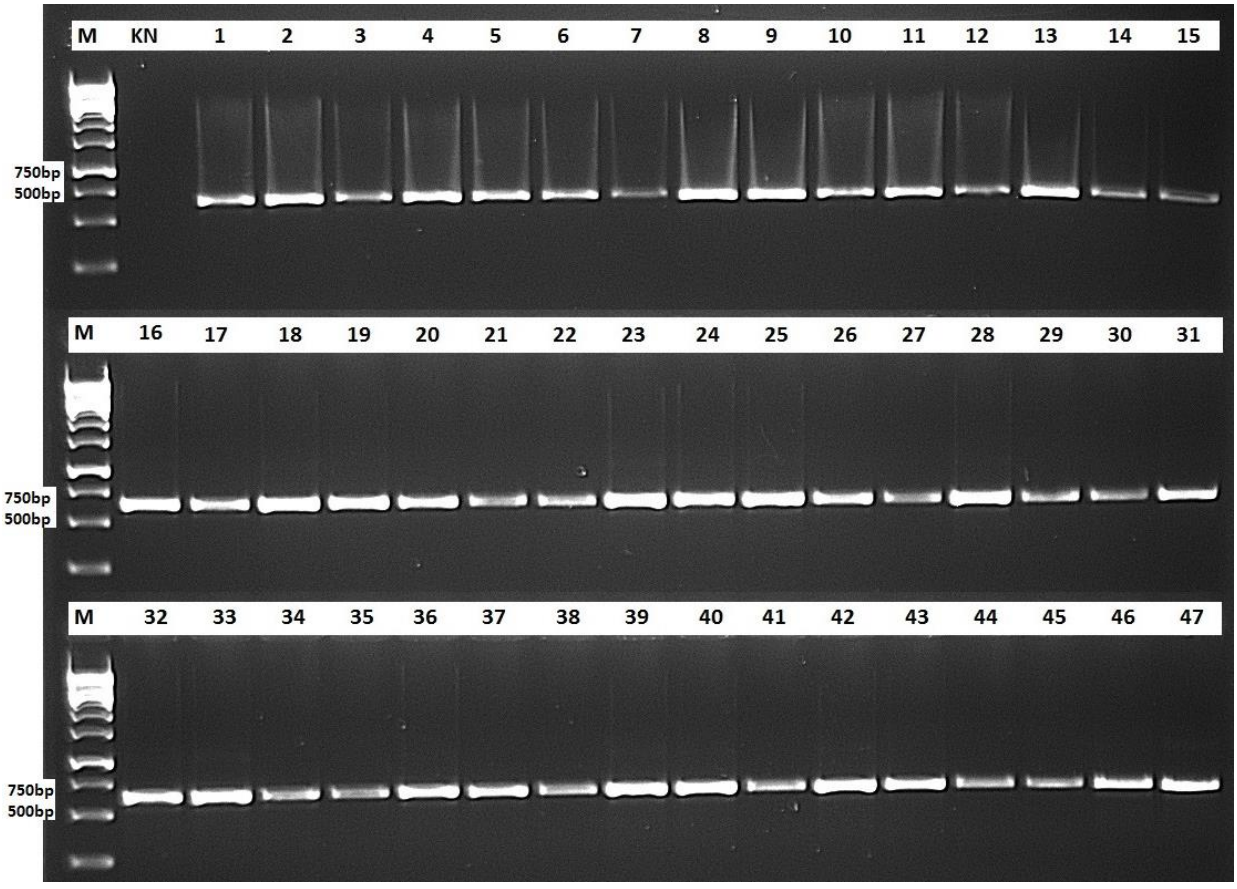

Fig. 4. Electrophorogram of PCR product amplified with the ITS1F and ITS4A primer set: 1–8 – DNA of *V. heterodoxa* isolates from highbush blueberry cv. Bluecrop, 9–29 – DNA of isolates obtained from highbush blueberry cv. Bluegold and two not known cultivars, 30–47 – DNA of isolates obtained from bilberry, M – marker GeneRuller 1 kb DNA Ladder ready to use (Fermentas), KN – negative control

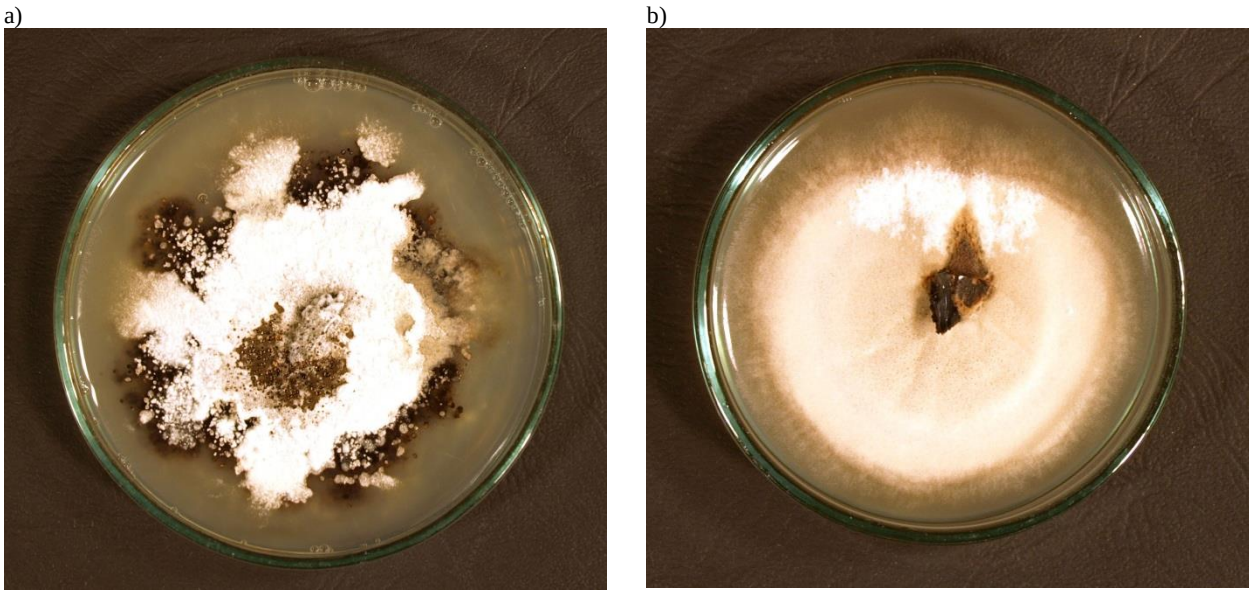

Fig. 5. Growth of isolates of *V. heterodoxa* on PDA: (a) group 1 and (b) group 2

Fig. 6. Primary conidium of the *V. heterodoxa* fungus from highbush blueberry infected leaf

Table 2. Comparison of conidia size of *V. heterodoxa* observed on different plant species

Plant species	Average size of conidia (µm)			
	Length of the arms		Width of the head	
Lingonberry	291.2	a*	103.1	a*
Bilberry	300.8	ab	128.8	b
Highbush blueberry cv. Bluecrop	314.2	b	138.3	b

* Letters indicate significant ($P < 0.05$) differences among means within each column, determined using Newman-Keuls test

The causal agent of leaf blight was identified on the basis of the molecular study as well. For all isolates mentioned above PCR products were about 520bp length (fig. 4). The nucleotide sequences of rDNA region (partial of 18S, complete ITS1, 5.8S, ITS2 and partial of 28S) of all 48 both obtained as reference isolates were identical. One of these sequences, obtained from bilberry isolates, was deposited in GenBank under the accession number KT121733.

The isolates showed diversity in mycelial morphology produced on PDA medium. Therefore, they were divided into two groups (fig. 5a, b). To the first group were classified isolates about a wavy myceli-

um both of highbush blueberry and bilberry. The colony was dark brown, gray or light gray, leathery and velvety. The edge of the colony was irregular. The reverse side was dark brown or black. With the aging of the cultures, the mycelium darkened and formed black spherical sclerotia. Isolates classified into the second group had a light cream mycelium with a shade of pink and gray. The central part of the colony was wrinkled, velvety, and leathery, and the edge of the colony was irregular. The reverse side was cream colored. The first group comprised 37 isolates, while only eleven isolates obtained from blueberry and bilberry belonged to the second group.

The best mycelium growth of the *V. heterodoxa* isolates both in highbush blueberry and bilberry was recorded on PDA medium. The cultures kept increasing by 2.76 mm per day. On OA there was only 2.42 mm per day on average of all isolates. Slightly less culture grew on WOA medium reaching a daily increase of 1.8 mm. The weaker growth of both groups of tested isolates was observed on SNA. Colonies were poorly developed, daily increase of 1.2 mm only was noted.

Sporulation of *V. heterodoxa* in laboratory condition was observed only on OA medium for isolates Vh/Un/1, Vh/Un/4 and Vh/J/2. The dimensions of conidia were 278 ±6 × 140 ±4 µm. Conidia observed on leaf spots of highbush blueberry, bilberry and lingonberry (fig. 6) were different in the length of the arms and the width of the head depending on the plant species they were collected from (tab. 2).

DISCUSSION

The subject of this study was the new aspects of the prevalence of the fungus *Valdensia heterodoxa*. Previous work published by Dzięcioł et al. [2014] concerned the occurrence of this fungus on highbush blueberry cv. Bluecrop grown in one of nursery plantations in the Mazovia province and described briefly its eight isolates only. In our study conducted in 2012–2015 we searched for symptoms of *V. heterodoxa* in different regions of Poland, on various plant species both in crop plantation, parks and forests. The research led to the identification of the fungus-infected leaves of *V. corymbosum* cv. Bluegold, two unknown cultivars of highbush blueberry and a dozen plants of bilberry. Similar spots and conidia on bilberry leaves were observed by Peyronel [1923], Redhead and Perrin [1972a], Redhead [1974], Holst-Jensen et al. [1997], Aamlid [2000], Magnussen et al. [2004], and Zhao and Shamoun [2010] and on leaves of highbush blueberry cv. Jersey by Nekoduka et al. [2012] in Japan and on cv. Bluecrop by Dzięcioł et al. [2014] in Poland. The pathogenicity of all forty obtained isolates of *V. heterodoxa* was confirmed. In our study symptoms which appeared on inoculated plants were seen only on young leaves, as in the case of observation conducted by Nekoduka et al. in 2012. However, in our investigation no sporulation of the fungus was observed, in contrast to the reports of Japanese scientists.

In our studies the same type of disease symptoms were observed on inoculated plants of bilberry and highbush blueberry regardless of the origin of isolates. These innovative studies suggest that the infected by *V. heterodoxa* fungus bilberry plants can be a source of infection for highbush blueberry.

Molecular characterization of all (40) isolates revealed high homogeneity between nucleotide sequences of the rDNA region. Alignment of those sequences to the ones deposited in GenBank of *V. heterodoxa* from Japan [Nekoduka et al. 2012], Norway [Holst-Jensen et al. 1997] and Poland [Dzięcioł et al. 2014] showed 100% similarity. The nucleotide sequences of bilberry isolates were obtained and describe for the first time in Poland in our studies. Their reference sequence (VH/J/2) was deposited in GenBank (accession no. KT121733).

In the available literature there is a lack of information relevant to the growth of *V. heterodoxa* on PDA medium. In our study isolates showed diversity in the mycelial morphology on this medium. Cultures were divided into two group.

Under natural conditions growth of the fungus depends on the weather during the growing season. Growth and development of *V. heterodoxa* and colonization of new plants by conidia require high humi-

dity. Similar conditions should be provided in the laboratory [Nekoduka et al. 2012]. Redhead and Perrin [1972a] noticed different growth of *V. heterodoxa* isolates depending on the medium and temperature. The highest sporulation of star-shaped conidia (primary conidia, aleuriospores) was observed on CMA (corn meal agar) and WOA at 15°C for 4–5 days. Secondary conidia (phialospores) developed on MYSA (malt-yeast-soytone agar) medium at 10°C. The optimum temperature for mycelial growth was 20°C with a 12-hour photoperiod. The fungus grew very slowly at 4°C, while at 30°C complete inhibition of growth was noticed. Vogelgsang and Shamoun [2002] confirmed that sporulation was highly dependent on the temperature and photoperiod. Both lower temperature (<10°C) and darkness (0-hour photoperiod) inhibited the growth and development of the fungus. Two isolates tested by these authors needed 17°C by day and 12°C by night with supplemental lighting for a 12-hour photoperiod for the best growth and sporulation. Magnussen et al. [2004] noted the best sporulation at a 12-hour photoperiod and temperature in the range from 16 to 19°C. Zhao and Shamoun [2006] showed that the best conditions for growth and sporulation was a temperature of 19°C (day) and 12°C (night) and a 12-hour photoperiod on media SPDA (salal-PDA), SOA (salal-oatmeal agar) and WOA. In our study the best growth of the two selected isolates of *V. heterodoxa* from highbush blueberry and bilberry was noted on PDA medium. Cultures formed compact, well-developed tallus. With time, sclerotia were formed on the surface of the cultures, likewise in the investigation of Redhead and Perrin [1972a] on MYSA medium (in the temperature 15–20°C after 7–14 days). On WOA colonies had long, thin and poorly developed hyphae. On SNA medium the growth was weaker than on other tested media. In contrast to the results obtained by Vogelgsang and Shamoun [2002], Magnussen et al. [2004], and Zhao and Shamoun [2006], it was found that cultures responded better to daylight than fluorescent lamp irradiation.

Zhao and Shamoun [2010] shown that the spore size depends on the type of medium. These researchers indicated that the fungus produced spores with a length of 238–389 μm on WOA, whereas on agar media with wheat bran and rice the average length of spores was significantly different. On the medium with wheat bran, the length of spores was 374 μm, while the spores from rice were relatively shorter (283 μm). The ratio of the length of the arms to the width of the head of spores was similar for all media. In our laboratory experiments sporulation of selected isolates of *V. heterodoxa* was observed on OA medium (isolates Vh/Un/1, Vh/Un/4 and Vh/J/2) only. Hyaline star-shaped conidia was formed in the central portion of culture. The size of these spores were 278 ±6 × 140 ±4 μm.

In natural conditions the size of conidia depended on the plant species which they were obtained from. Conidia were different in the length of the arms and width of the head. The highest average arm length was noted in conidia collected from infected highbush blueberry plants, while the shortest ones on lingonberry. The length of the conidia arms mentioned above were similar to those observed by Bavendamm [1944] (200–275 μm) but smaller than those observed by Mułenko and Woodward [1996] 400–600 μm in diameter.

CONCLUSIONS

1. In our investigation conducted in 2012–2015 *V. heterodoxa* was observed commonly on leaves of wild-growing bilberry in Lublin, Pomerania and Podlasie provinces.

2. The symptoms of this disease on highbush blueberry and bilberry plants were noted in July, August, and September. Also lingonberry plants were infected. Symptoms on this plant species were observed in Lublin only.

3. The size of conidia *V. heterodoxa* depended on the plant species which they were obtained from.

4. Isolates showed diversity in the mycelial morphology on different media.

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